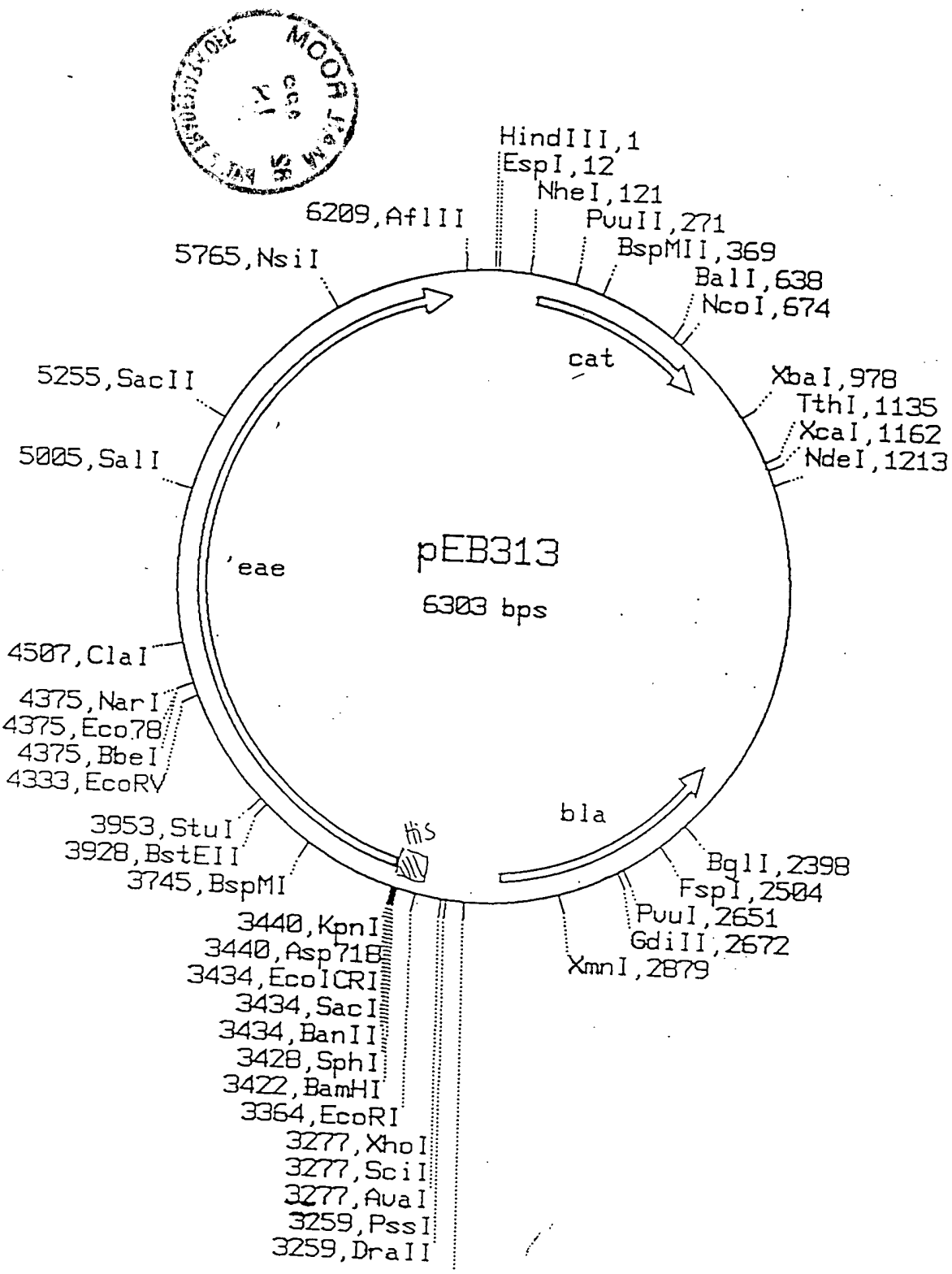
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NheI,121
PuuII,271
BspMII,369
BalI,638
NcoI,674
XbaI,978
TthI,1135
XcaI,1162
NdeI,1213
Bgl I,2398
FspI,2504
PuuI,2651
GdiII,2672
XmnI,2879
3440,KpnI
3440,Asp718
3434,EcoICRI
3434,SacI
3434,BanII
3428,SphI
3422,BamHI
3364,EcoRI
3277,XhoI
3277,SciI
3277,AvaI
3259,PssI
3259,DraII
3953,StuI
3928,BstEII
3745,BspMI
4507,ClaI
4375,NarI
4375,Eco78
4375,BbeI
4333,EcoRV
5005,SalI
5255,SacII
5765,NsiI
6209,AflII
cat
bla
his
'eae
pEB313
6303 bps

FIGURE 1

MOORE BPA

```
  1  MITHGCYTRT RHKHKLKKTL IMLSAGLGLF FYVNQNSFAN GENYFKLGSD
 51  SKLLTHDSYQ NRLFYTLKTG ETVADLSKSQ DINLSTIWSL NKHLYSSESE
101  MMKAAPGQQI ILPLKKLPFE YSALPLLGSA PLVAAGGVAG HTNKLTKMSP
151  DVTKSNMTDD KALNYAAQQA ASLGSQLQSR SLNGDYAKDT ALGIAGNQAS
201  SQLQAWLQHY GTAEVNLQSG DNFDGSSLDF LLPFYDSEKM LAFGQVGARY
251  IDSRFTANLG AGQRFFLPAN MLGYNVFIDQ DFSGDNTRLG IGGEYWRDYF
301  KSSVNGYFRM RRWHESYHKK DYDERPANGF DIRFNGYLPS YPALGAKLIY
351  EQYYGDNVAL FNSDKLQSNP GAATVGVNYT PIPLVTMGID YRHGTGNEND
401  LLYSMQFRYQ FDKSWSQQIE PQYVNELRTL SGSRYDLVQR NNNIILEYKK
451  QDILSLNIPH DINGTEHSTQ KIQLIVKSKY GLDRIVWDDS ALRSQGGQIQ
501  HSGSQSAQDY QAILPAYVQG GSNIYKVTAR AYDRNGNSSN NVQLTITVLS
551  NGQVVDQVGV TDFTADKTSA KADNADTITY TATVKKNGVA QANVPVSFNI
601  VSGTATLGAN SAKTDANGKA TVTLKSSTPG QVVVSAKTAE MSSALNASAV
651  IFFDQTKASI TEIKADKTTA VANGKDAIKY TVKVMKNGQP VNNQSVTFST
701  NFGMFNGKSQ TQATTGNDGR ATITLTSSSA GKATVSATVS DGAEVKATEV
751  TFFDELKIDN KVDIIGNNVR GELPNIWLQY GQFKLKASGG DGTYSWYSEN
801  TSIATVDASG KVTLNGKGSV VIKATSGDKQ TVSYTIKAPS YMIKVDKQAY
851  YADAMSICKN LLPSTQTVLS DIYDSWGAAN KYSHYSSMNS ITAWIKQTSS
901  EQRSGVSSTY NLITQNPLPG VNVNTPNVYA VCVE
```

FIGURE 2

```
   1  TCGAGAATGA AATAGAAGTC GTTGTTAAGT CAATGGAAAA CCTGTATTTG GTATTACATA
  61  ATCAGGGAAT AACATTAGAA AACGAACATA TGAAAATAGA GGAAATCAGT TCAAGCGACA
 121  ATAAACATTA TTACGCCGGA AGATAAAATC CGATCTATTA ATATAATTTA TTTCTCATTC
 181  TAACTCATTG TGGTGGAGCC ATAACATGAT TACTCATGGT TGTTATACCC GGACCCGGCA
 241  CAAGCATAAG CTAAAAAAAA CATTGATTAT GCTTAGTGCT GGTTTAGGAT TGTTTTTTTA
 301  TGTTAATCAG AATTCATTTG CAAATGGTGA AAATTATTTT AAATTGGGTT CGGATTCAAA
 361  ACTGTTAACT CATGATAGCT ATCAGAATCG CCTTTTTTAT ACGTTGAAAA CTGGTGAAAC
 421  TGTTGCCGAT CTTTCTAAAT CGCAAGATAT TAATTTATCG ACGATTTGGT CGTTGAATAA
 481  GCATTTATAC AGTTCTGAAA GCGAAATGAT GAAGGCCGCG CCTGGTCAGC AGATCATTTT
 541  GCCACTCAAA AAACTTCCCT TTGAATACAG TGCACTACCA CTTTTAGGTT CGGCACCTCT
 601  TGTTGCTGCA GGTGGTGTTG CTGGTCACAC GAATAAACTG ACTAAAATGT CCCCGGACGT
 661  GACCAAAAGC AACATGACCG ATGACAAGGC ATTAAATTAT GCGGCACAAC AGGCGGCGAG
 721  TCTCGGTAGC CAGCTTCAGT CGCGATCTCT GAACGGCGAT TACGCGAAAG ATACCGCTCT
 781  TGGTATCGCT GGTAACCAGG CTTCGTCACA GTTGCAGGCC TGGTTACAAC ATTATGGAAC
 841  GGCAGAGGTT AATCTGCAGA GTGGTAATAA CTTTGACGGT AGTTCACTGG ACTTCTTATT
 901  ACCGTTCTAT GATTCCGAAA AAATGCTGGC ATTTGGTCAG GTCGGAGCGC GTTACATTGA
 961  CTCCCGCTTT ACGGCAAATT TAGGTGCGGG TCAGCGTTTT TTCCTTCCTG CAAACATGTT
1021  GGGCTATAAC GTCTTCATTG ATCAGGATTT TTCTGGTGAT AATACCCGTT TAGGTATTGG
1081  TGGCGAATAC TGGCGAGACT ATTTCAAAAG TAGCGTTAAC GGCTATTTCC GCATGAGCGG
1141  CTGGCATGAG TCATACAATA AGAAAGACTA TGATGAGCGC CCAGCAAATG GCTTCGATAT
1201  CCGTTTTAAT GGCTATCTAC CGTCATATCC GGCATTAGGC GCCAAGCTGA TATATGAGCA
1261  GTATTATGGT GATAATGTTG CTTTGTTTAA TTCTGATAAG CTGCAGTCGA ATCCTGGTGC
1321  GGCGACCGTT GGTGTAAACT ATACTCCGAT TCCTCTGGTG ACGATGGGGA TCGATTACCG
1381  TCATGGTACG GGTAATGAAA ATGATCTCCT TTACTCAATG CAGTTCCGTT ATCAGTTTGA
1441  TAAATCGTGG TCTCAGCAAA TTGAACCACA GTATGTTAAC GAGTTAAGAA CATTATCAGG
1501  CAGCCGTTAC GATCTGGTTC AGCGTAATAA CAATATTATT CTGGAGTACA AGAAGCAGGA
1561  TATTCTTTCT CTGAATATTC CGCATGATAT TAATGGTACT GAACACAGTA CGCAGAAGAT
1621  TCAGTTGATC GTTAAGAGCA AATACGGTCT GGATCGTATC GTCTGGGATG ATAGTGCATT
1681  ACGCAGTCAG GGCGGTCAGA TTCAGCATAG CGGAAGCCAA AGCGCACAAG ACTACCAGGC
1741  TATTTTGCCT GCTTATGTGC AAGGTGGCAG CAATATTTAT AAAGTGACGG CTCGCGCCTA
1801  TGACCGTAAT GGCAATAGCT CTAACAATGT ACAGCTTACT ATTACCGTTC TGTCGAATGG
1861  TCAAGTTGTC GACCAGGTTG GGGTAACGGA CTTTACGGCG GATAAGACTT CGGCTAAAGC
1921  GGATAACGCC GATACCATTA CTTATACCGC GACGGTGAAA AAGAATGGGG TAGCTCAGGC
1981  TAATGTCCCT GTTTCATTTA ATATTGTTTC AGGAACTGCA ACTCTTGGGG CAAATAGTGC
2041  CAAAACGGAT GCTAACGGTA AGGCAACCGT AACGTTGAAG TCGAGTACGC CAGGACAGGT
2101  CGTCGTGTCT GCTAAAACCG CGGAGATGAC TTCAGCACTT AATGCCAGTG CGGTTATATT
2161  TTTTGATCAA ACCAAGGCCA GCATTACTGA GATTAAGGCT GATAAGACAA CTGCAGTAGC
2221  AAATGGTAAG GATGCTATTA AATATACTGT AAAAGTTATG AAAAACGGTC AGCCAGTTAA
2281  TAATCAATCC GTTACATTCT CAACAAACTT TGGGATGTTC AACGGTAAGT CTCAAACGCA
2341  AGCAACCACG GGAAATGATG GTCGTGCGAC GATAACACTA ACTTCCAGTT CCGCCGGTAA
2401  AGCGACTGTT AGTGCGACAG TCAGTGATGG GGCTGAGGTT AAAGCGACTG AGGTCACTTT
2461  TTTTGATGAA CTGAAAATTG ACAACAAGGT TGATATTATT GGTAACAATG TCAAGAGGTC
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2641  AGTCACTTTG AATGGTAAAG GCAGTGTCGT AATTAAAGCC ACATCTGGTG ATAAGCAAAC
2701  AGTAAGTTAC ACTATAAAAG CACCGTCGTA TATGATAAAA GTGGATAAGC AAGCCTATTA
2761  TGCTGATGCT ATGTCCATTT GCAAAAATTT ATTACCATCC ACACAGACGG TATTGTCAGA
2821  TATTTATGAC TCATGGGGGG CTGCAAATAA ATATAGCCAT TATAGTTCTA TGAACTCAAT
2881  AACTGCTTGG ATTAAACAGA CATCTAGTGA GCAGCGTTCT GGAGTATCAA GCACTTATAA
2941  CCTAATAACA CAAAACCCTC TTCCTGGGGT TAATGTTAAT ACTCCAAATG TCTATGCGGT
3001  TTGTGTAGAA TAATTCCATA ACCACCCCGG CTAAAATATG TATTGTTTTA GTCGGGGCAT
3061  AATTATTTCT TCTTAAGAAA TAACCCTCTT ATAATCAAAT CTACTACTGG TCTTTTTATC
3121  TGCTTAATAG G
```

FIGURE 3

```
   1  GGAAAGATAA ATCCGATCTA TTAATATAAT TTATTTCTCA TTCTAACTCA TTGTGGTGGA
  61  GCCATAACAT GAGTACTCAT GGTTGTTATA CCCGGACCCG GCACAAGCAT AAGCTAAAAA
 121  AAACATTGAT TATGCTTAGT GCTGGTTTAG GATTGTTTTT TTATGTTAAT CAGAATTCAT
 181  TTGCAAATGG TGAAAATTAT TTTAAATTGG GTTCGGATTC AAAACTGTTA ACTCATGATA
 241  GCTATCAGAA TCGCCTTTTT TATACGTTGA AAACTGGTGA AACTGTTGCC GATCTTTCTA
 301  AATCGCAAGA TATTAATTTA TCGACGATTT GGTCGTTGAA TAAGCATTTA TACAGTTCTG
 361  AAAGCGAAAT GATGAAGGCC GCGCCTGGTC AGCAGATCAT TTTGCCACTC AAAAAACTTC
 421  CCTTTGAATA CAGTGCACTA CCACTTTTAG GTTCGGCACC TCTTGTTGCT GCAGGTGGTG
 481  TTGCTGGTCA CACGAATAAA CTGACTAAAA TGTCCCCGGA CGTGACCAAA AGCAACATGA
 541  CCGATGACAA GGCATTAAAT TATGCGGCAC AACAGGCGGC GAGTCTCGGT AGCCAGCTTC
 601  AGTCGCGATC TCTGAACGGC GATTACGCGA AAGATACCGC TCTTGGTATC GCTGGTAACC
 661  AGGCTTCGTC ACAGTTGCAG GCCTGGTTAC AACATTATGG AACGGCAGAG GTTAATCTGC
 721  AGAGTGGTGA TAACTTTGAC GGTAGTTCAC TGGACTTCTT ATTACCGTTC TATGATTCCG
 781  AAAAAATGCT GGCATTTGGT CAGGTCGGAG CGCGTTACAT TGACTCCCGC TTTACGGCAA
 841  ATTTAGGTGC GGGTCAGCGT TTTTTCCTTC CTGCAAACAT GTTGGGCTAT AACGTCTTCA
 901  TTGATCAGGA TTTTTCTGGT GATAATACCC GTTTAGGTAT TGGTGGCGAA TACTGGCGAG
 961  ACTATTTCAA AAGTAGCGTT AACGGCTATT TCCGCATGAG GCGCTGGCAT GAGTCATACC
1021  ATAAGAAAGA CTATGATGAG CGCCCAGCAA ATGGCTTCGA TATCCGTTTT AATGGCTATC
1081  TACCGTCATA TCCGGCATTA GGCGCCAAGC TGATATATGA GCAGTATTAT GGTGATAATG
1141  TTGCTTTGTT TAATTCTGAT AAGCTGCAGT CGAATCCTGG TGCGGCGACC GTTGGTGTAA
1201  ACTATACTCC GATTCCTCTG GTGACGATGG GGATCGATTA CCGTCATGGT ACGGGTAATG
1261  AAAATGATCT CCTTTACTCA ATGCAGTTCC GTTATCAGTT TGATAAATCG TGGTCTCAGC
1321  AAATTGAACC ACAGTATGTT AACGAGTTAA GAACATTATC AGGCAGCCGT TACGATCTGG
1381  TTCAGCGTAA TAACAATATT ATTCTGGAGT ACAAGAAGCA GGATATTCTT TCTCTGAATA
1441  TTCCGCATGA TATTAATGGT ACTGAACACA GTACGCAGAA GATTCAGTTG ATCGTTAAGA
1501  GCAAATACGG TCTGGATCGT ATCGTCTGGG ATGATAGTGC ATTACGCAGT CAGGGCGGTC
1561  AGATTCAGCA TAGCGGAAGC CAAAGCGCAC AAGACTACCA GGCTATTTTG CCTGCTTATG
1621  TGCAAGGTGG CAGCAATATT TATAAAGTGA CGGCTCGCGC CTATGACCGT AATGGCAATA
1681  GCTCTAACAA TGTACAGCTT ACTATTACCG TTCTGTCGAA TGGTCAAGTT GTCGACCAGG
1741  TTGGGGTAAC GGACTTTACG GCGGATAAGA CTTCGGCTAA AGCGGATAAC GCCGATACCA
1801  TTACTTATAC CGCGACGGTG AAAAAGAATG GGGTAGCTCA GGCTAATGTC CCTGTTTCAT
1861  TTAATATTGT TTCAGGAACT GCAACTCTTG GGGCAAATAG TGCCAAAACG GATGCTAACG
1921  GTAAGGCAAC CGTAACGTTG AAGTCGAGTA CGCCAGGACA GGTCGTCGTG TCTGCTAAAA
1981  CCGCGGAGAT GAGTTCAGCA CTTAATGCCA GTGCGGTTAT ATTTTTTGAT CAAACCAAGG
2041  CCAGCATTAC TGAGATTAAG GCTGATAAGA CAACTGCAGT AGCAAATGGT AAGGATGCTA
2101  TTAAATATAC TGTAAAAGTT ATGAAAAACG GTCAGCCAGT TAATAATCAA TCCGTTACAT
2161  TCTCAACAAA CTTTGGGATG TTCAACGGTA AGTCTCAAAC GCAAGCAACC ACGGGAAATG
2221  ATGGTCGTGC GACGATAACA CTAACTTCCA GTTCCGCCGG TAAAGCGACT GTTAGTGCGA
2281  CAGTCAGTGA TGGGGCTGAG GTTAAAGCGA CTGAGGTCAC TTTTTTTGAT GAACTGAAAA
2341  TTGACAACAA GGTTGATATT ATTGGTAACA ATGTCAGAGG CGAGTTGCCT AATATTTGGC
2401  TGCAATATGG TCAGTTTAAA CTGAAAGCAA GCGGTGGTGA TGGTACATAT TCATGGTATT
2461  CAGAAAATAC CAGTATCGCG ACTGTCGATG CATCAGGGAA AGTCACTTTG AATGGTAAAG
2521  GCAGTGTCGT AATTAAAGCC ACATCTGGTG ATAAGCAAAC AGTAAGTTAC ACTATAAAAG
2581  CACCGTCGTA TATGATAAAA GTGGATAAGC AAGCCTATTA TGCTGATGCT ATGTCCATTT
2641  GCAAAAATTT ATTACCATCC ACACAGACGG TATTGTCAGA TATTTATGAC TCATGGGGGG
2701  CTGCAAATAA ATATAGCCAT TATAGTTCTA TGAACTCAAT AACTGCTTGG ATTAAACAGA
2761  CATCTAGTGA GCAGCGTTCT GGAGTATCAA GCACTTATAA CCTAATAACA CAAAACCCTC
2821  TTCCTGGGGT TAATGTTAAT ACTCCAAATG TCTATGCGGT TTGTGTAGAA TAATTCCATA
2881  ACCACCCCGG CTAAAATATG TATTGTTTTA GTCGGGGCAT AATTATTTCT TCTTAAGAAA
2941  TAACCTCTTA TAATCAAATC TACTACTGGT CTTTTTATCT GCTTAATAGG TCTCTTTCAA
3001  AGAGACACAT TCACGTTTTC TAGAGTAGGT TGATCCAACC ACGCTGTATA CCAAAGCTGA
3061  ATCACATCAA GCAACAACTA TGCTCACAAC ATCCACACAA TAAAAA
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FIGURE 4

MOOR JAMES

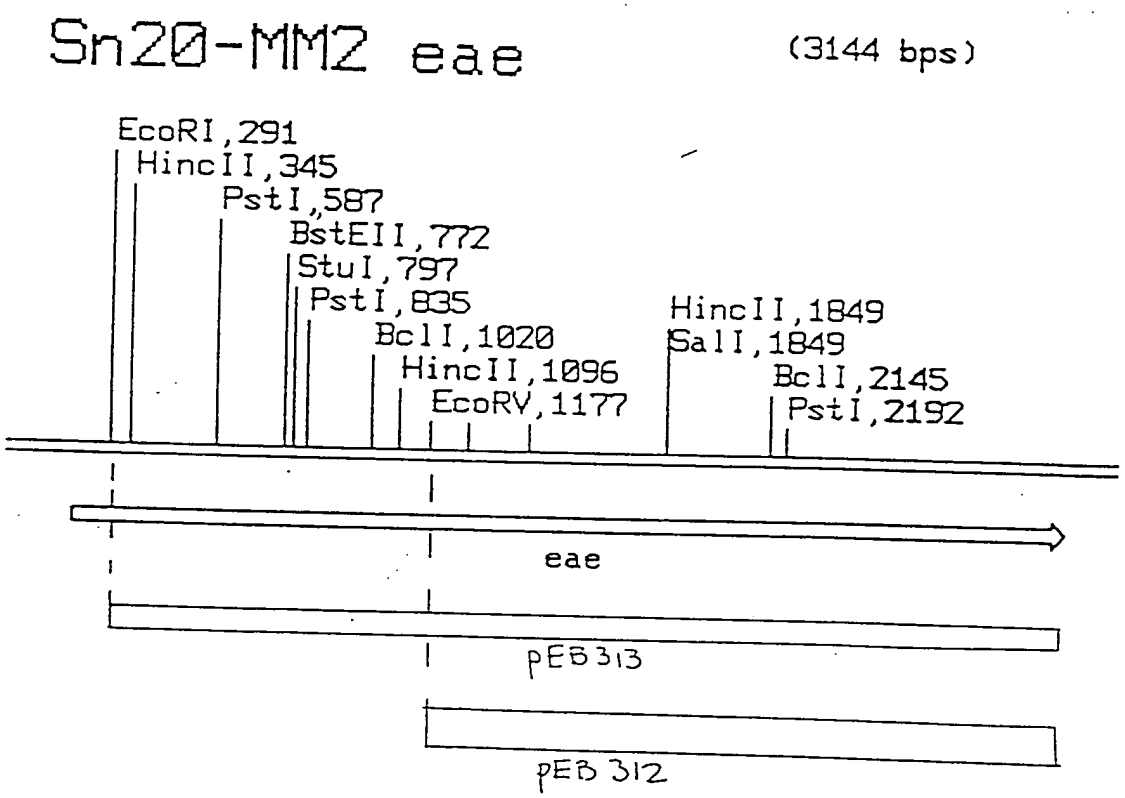



FIGURE 5

SUBSTITUTE SHEET (RULE 26)

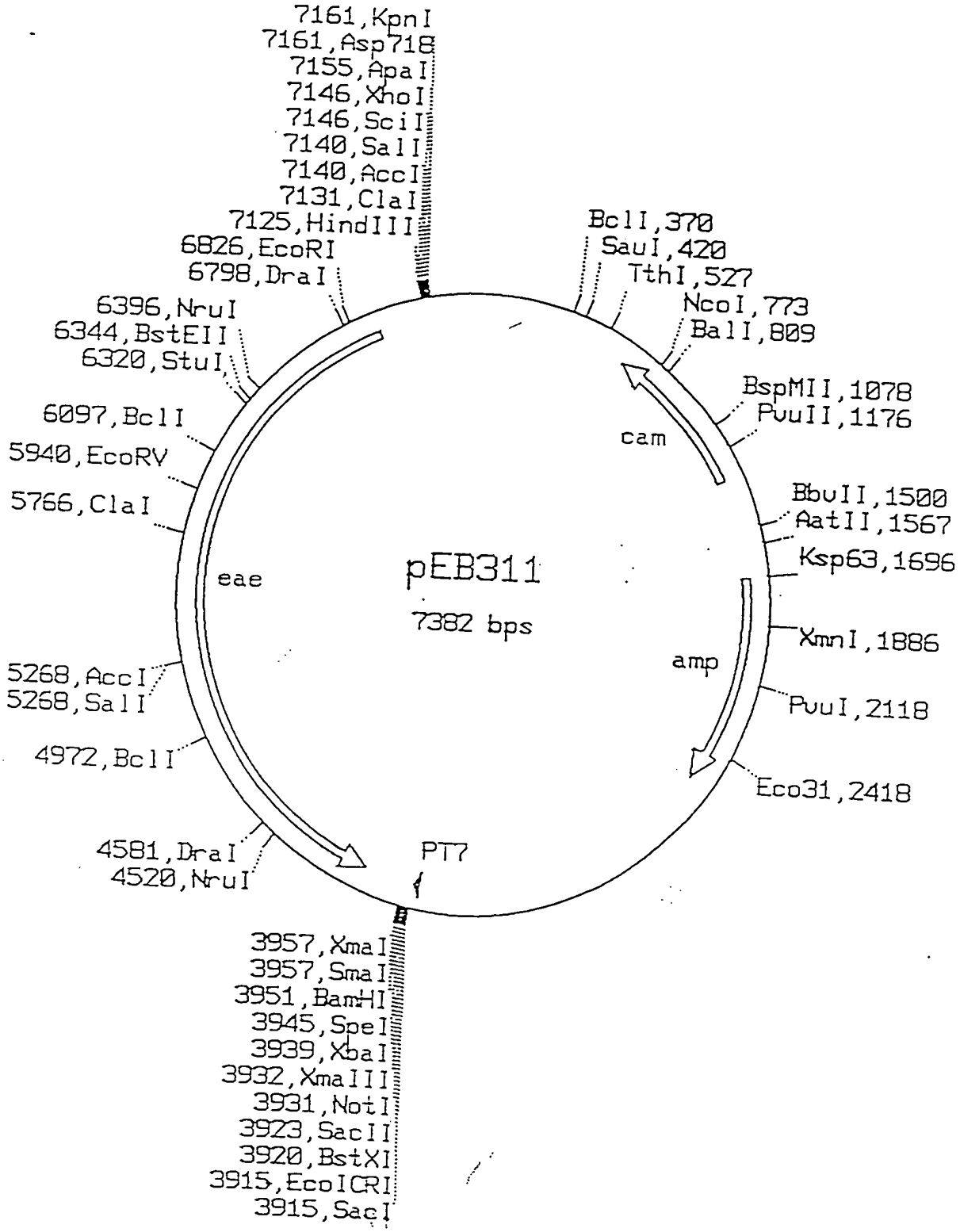
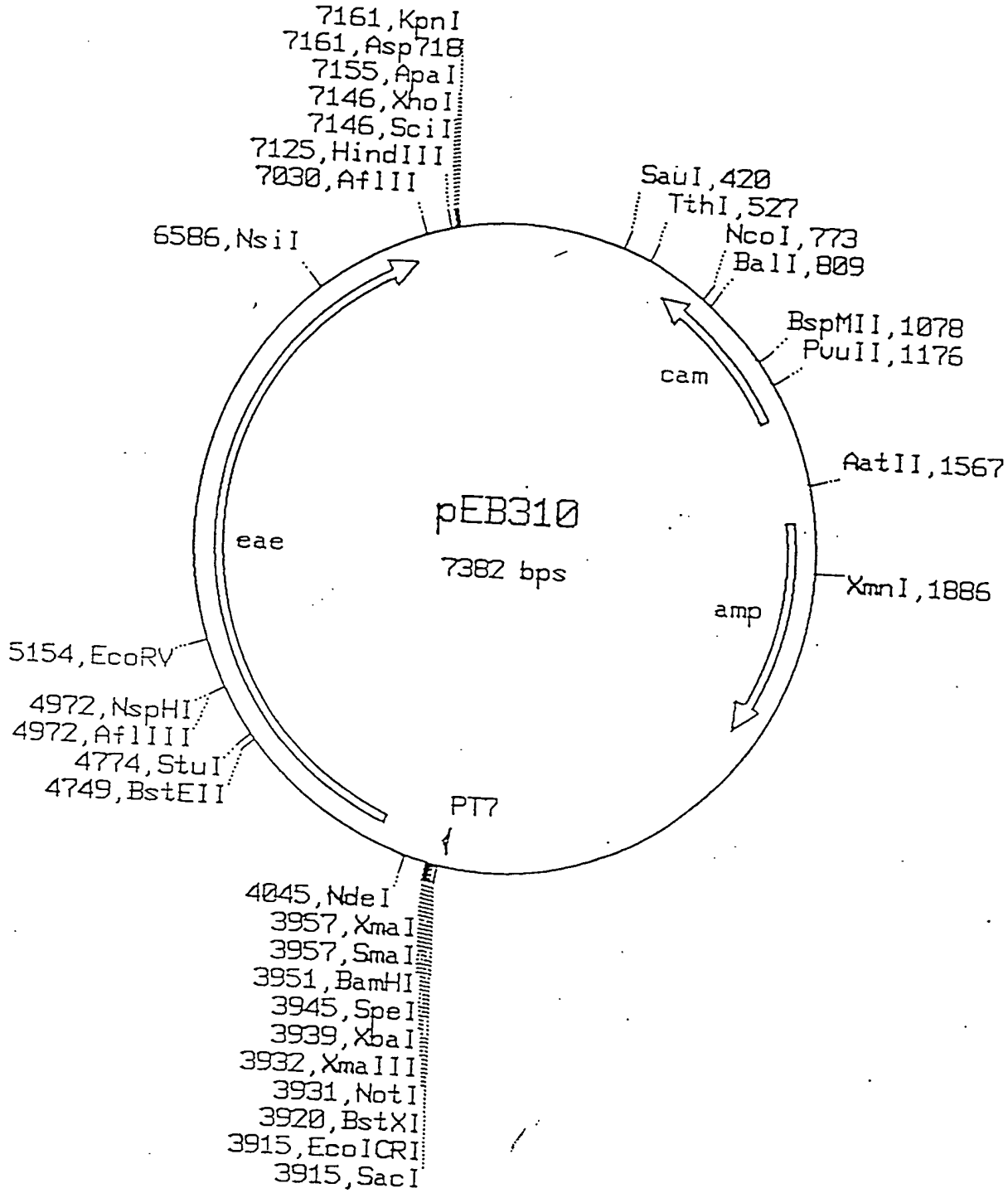



FIGURE 6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7146,XhoI
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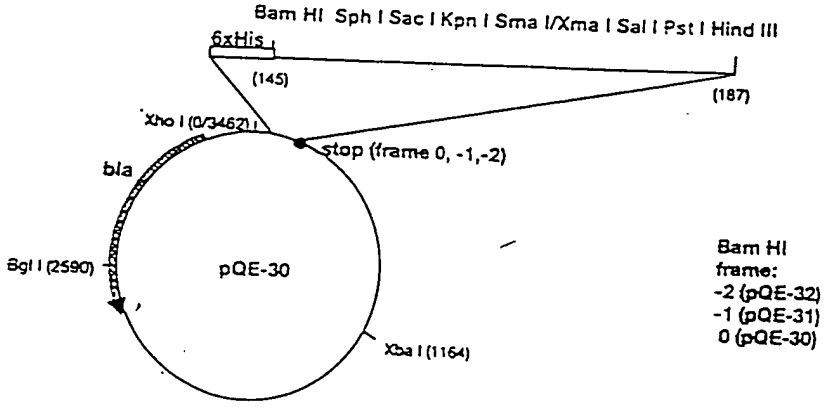
7125,HindIII
7030,AflII
6586,NsiI
SauI,420
TthI,527
NcoI,773
BalI,809
BspMII,1078
PvuII,1176
cam
AatII,1567
pEB310
7382 bps
eae
amp
XmnI,1886
5154,EcoRV
4972,NspHI
4972,AflIII
4774,StuI
4749,BstEII
PT7
4045,NdeI
3957,XmaI
3957,SmaI
3951,BamHI
3945,SpeI
3939,XbaI
3932,XmaIII
3931,NotI
3920,BstXI
3915,EcoICRI
3915,SacI

FIGURE 7


Bam HI Sph I Sac I Kpn I Sma I/Xma I Sal I Pst I Hind III
6xHis
(145)
(187)
Xho I (0/3462)
stop (frame 0, -1,-2)
bla
Bgl I (2590)
pQE-30
Xba I (1164)
Bam HI
frame:
-2 (pQE-32)
-1 (pQE-31)
0 (pQE-30)

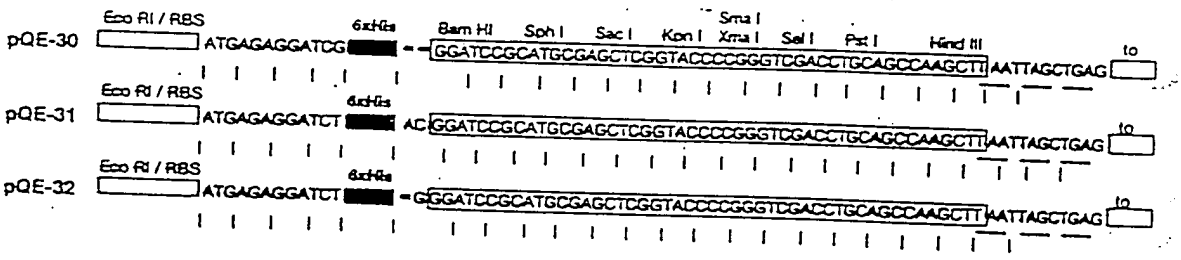
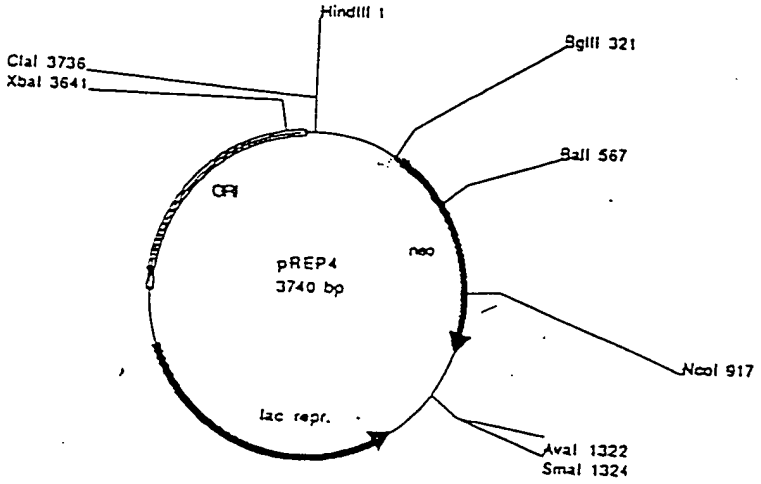

Eco RI / RBS
6xHis
Bam HI
Sph I
Sac I
Kpn I
Sma I
Xma I
Sal I
Pst I
Hind III
pQE-30
ATGAGAGGATCG
GGATCCGCATGCGAGCTCGGTACCCCGGGTCGACCTGCAGCCAAGCTT
AATTAGCTGAG
to
pQE-31
ATGAGAGGATCT
AC GGATCCGCATGCGAGCTCGGTACCCCGGGTCGACCTGCAGCCAAGCTT
AATTAGCTGAG
to
pQE-32
ATGAGAGGATCT
G GGATCCGCATGCGAGCTCGGTACCCCGGGTCGACCTGCAGCCAAGCTT
AATTAGCTGAG
to

FIGURE 8




```
                Xho I                                                operator I
  1  CTCGAGAAAT CATAAAAAAT TTATTTGCTT TGTGAGCGGA TAACAATTAT
                                                                    TATA-Box
                          operator II                 Eco RI
 51  AATAGATTCA ATTGTGAGCG GATAACAATT TCACACAGAA TTCATTAAAG
                 +1 start mRNA
                          ATG →                 6xHis
101  AGGAGAAATT AACTATGAGA GGATCGCATC ACCATCACCA TCACGGATCC
     RBS/SD                                                  Bam HI

151  GCATGCGAGC TCGGTACCCC GGGTCGACCT GCAGCCAAGC TTAATTAGCT
       Sph I  Sac I  Kpn I  Sma I  Sal I  Pst I  Hind III  — — —
                                                  Stop 1 2 3

201  GAGCTTGGAC TCCTGTTGAT AGATCCAGTA ATGACCTCAG AACTCCATCT
     —
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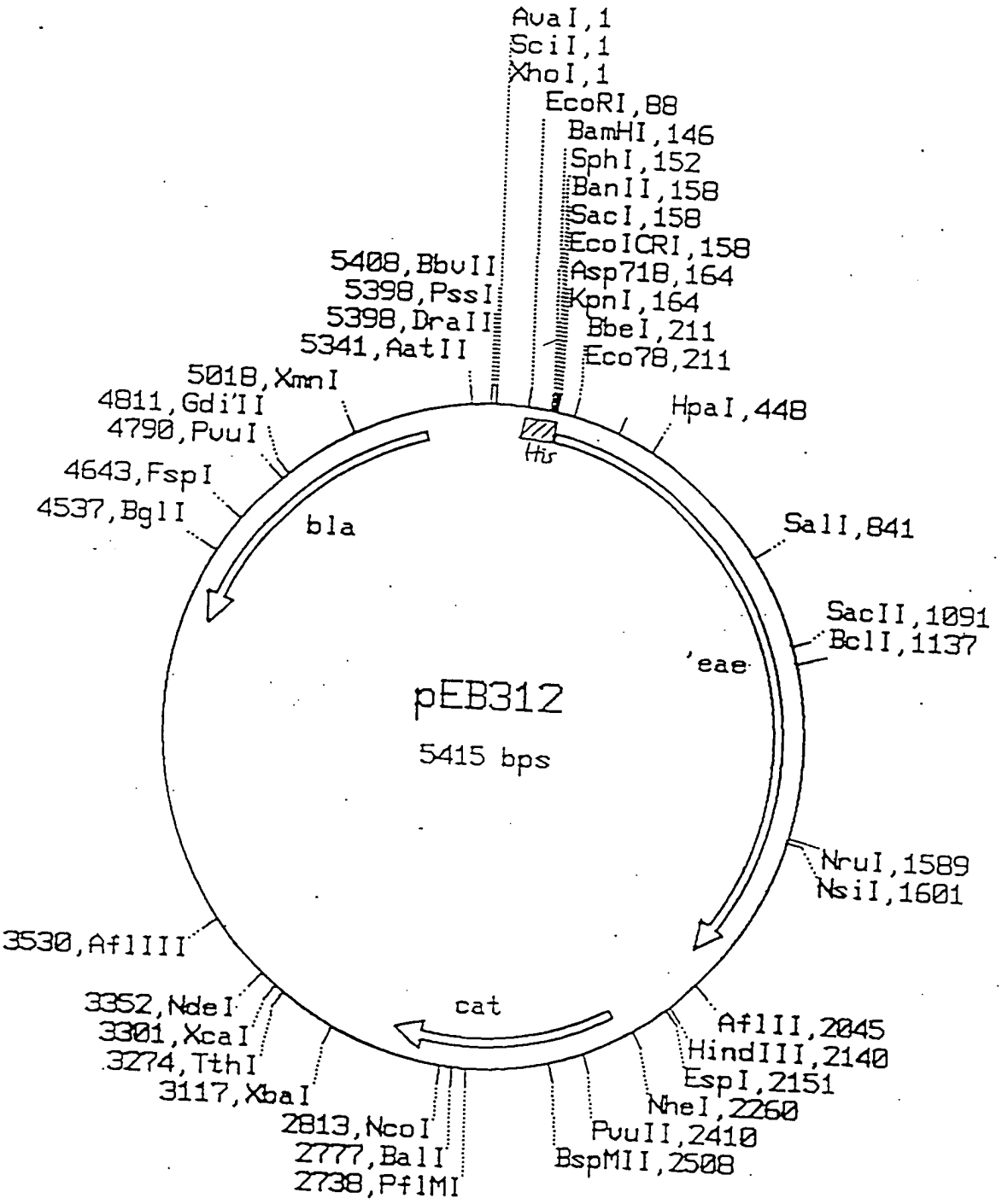
FIGURE 9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SciI,1
XhoI,1
EcoRI,88
BamHI,146
SphI,152
BanII,158
SacI,158
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Asp718,164
KpnI,164
BbeI,211
Eco78,211
HpaI,448
SalI,841
SacII,1091
BclI,1137
NruI,1589
NsiI,1601
AflII,2045
HindIII,2140
EspI,2151
NheI,2260
PvuII,2410
BspMII,2508
2813,NcoI
2777,BalI
2738,Pf1MI
3117,XbaI
3274,TthI
3301,XcaI
3352,NdeI
3530,AflIII
4537,BglI
4643,FspI
4790,PvuI
4811,GdiII
5018,XmnI
5341,AatII
5398,DraII
5398,PssI
5408,BbvII
His
bla
'eae
cat
pEB312
5415 bps

FIGURE 10

2687402 05/25/99

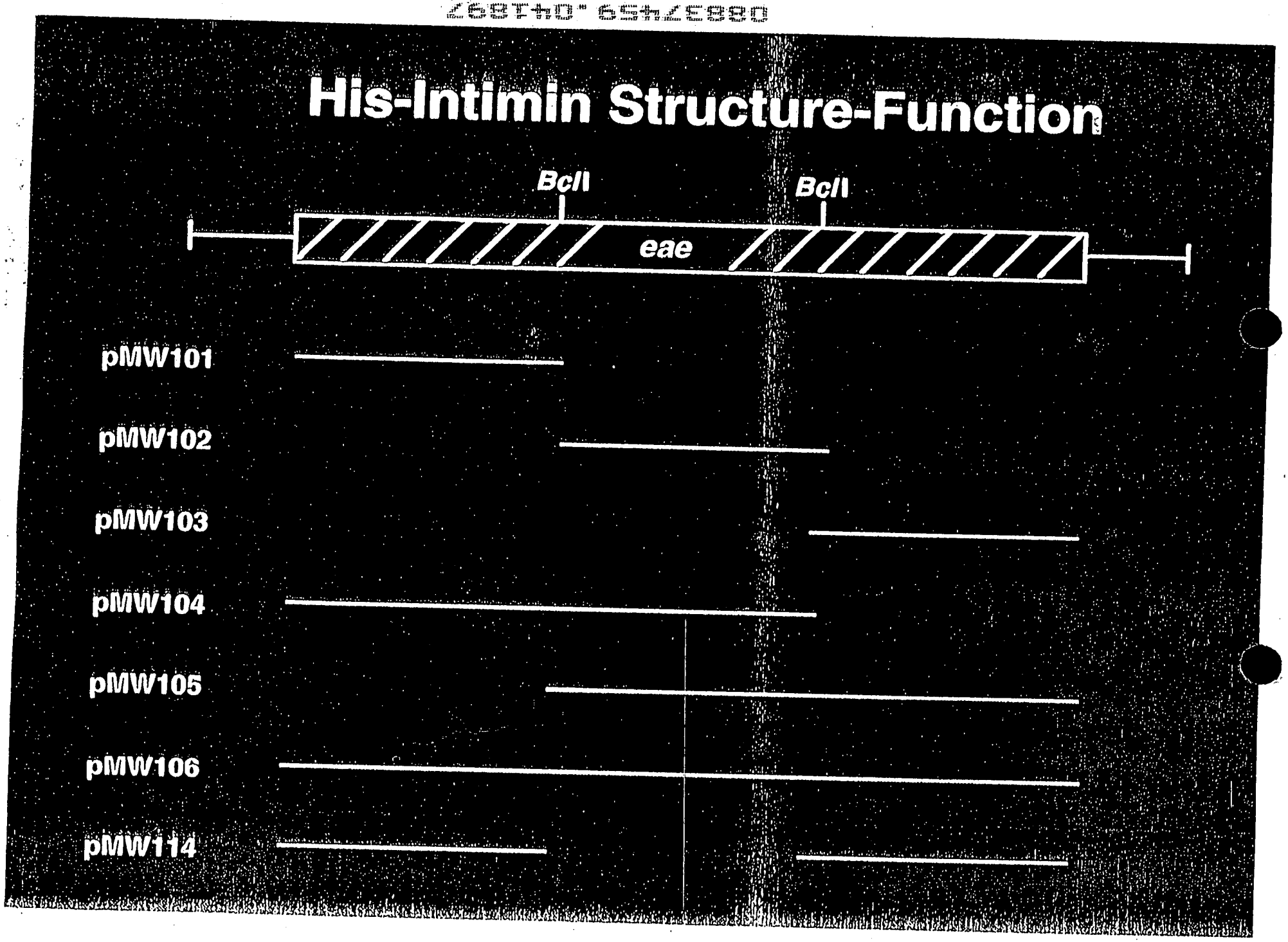



FIGURE 11

2687+0 6S+2E980

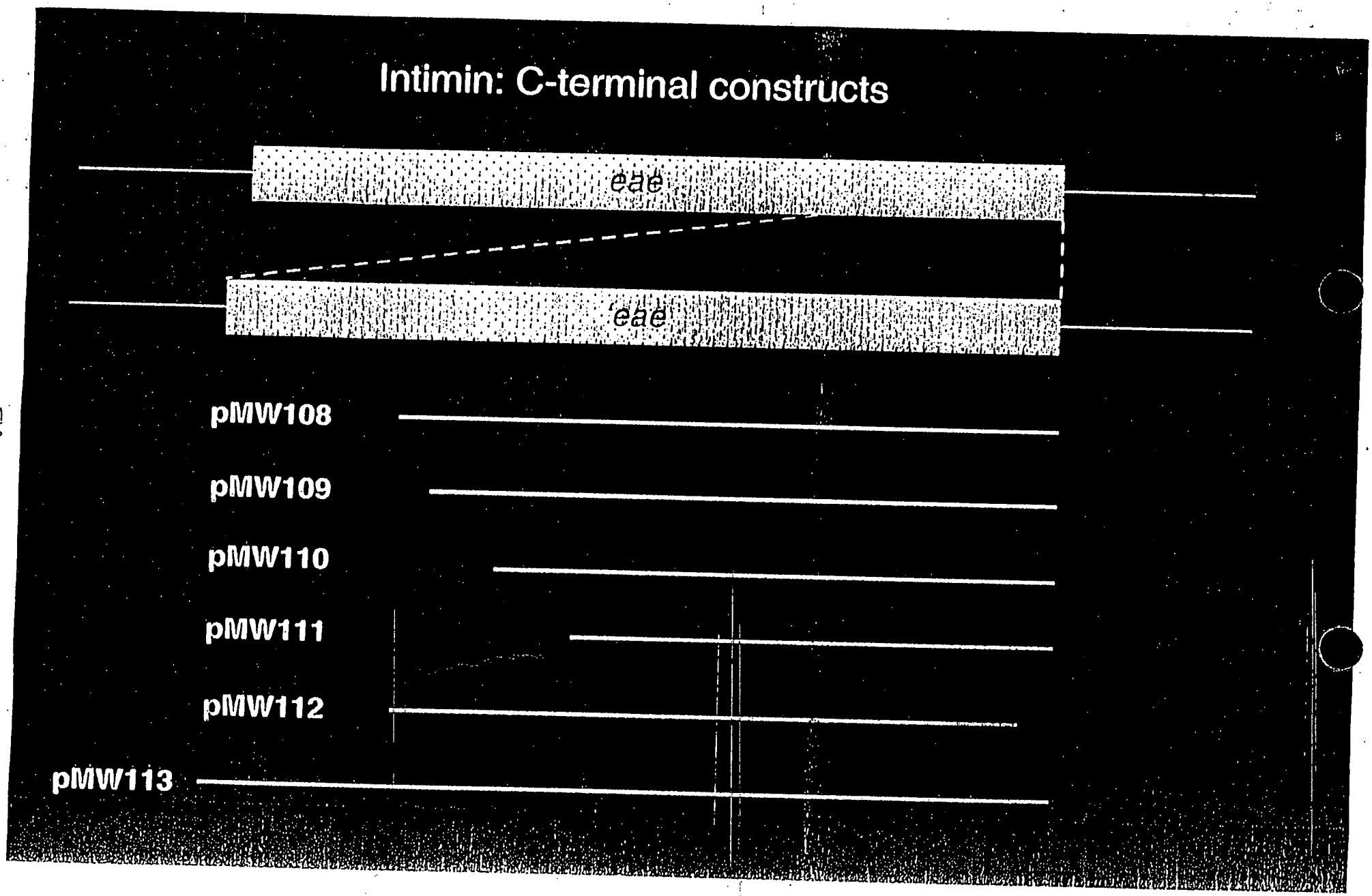



FIGURE 12

2687HO" 6S+2S880

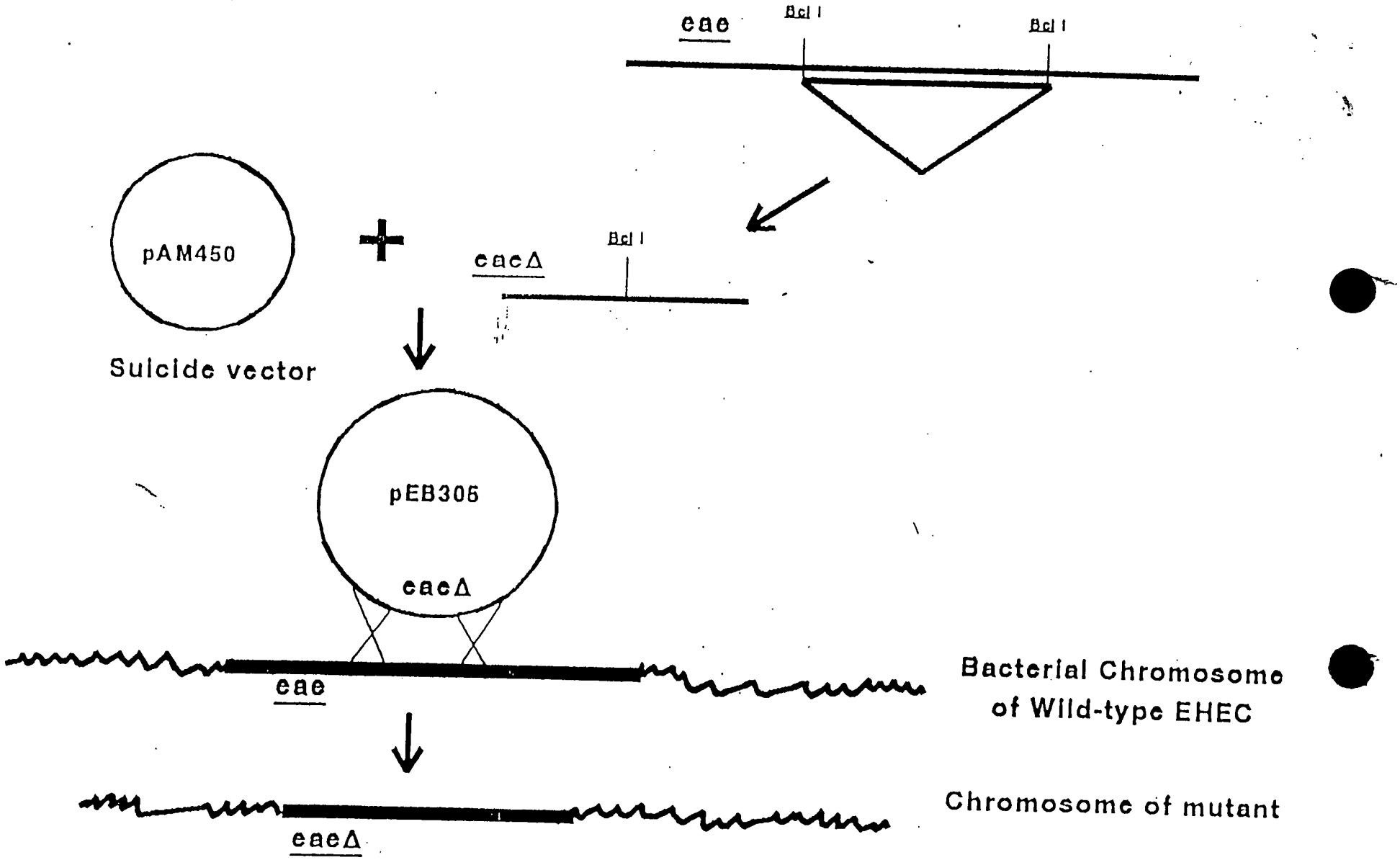



FIGURE 13

MOOR JAMES & THOMPSON
RECEIVED
JAN 28
PATENT OFFICE

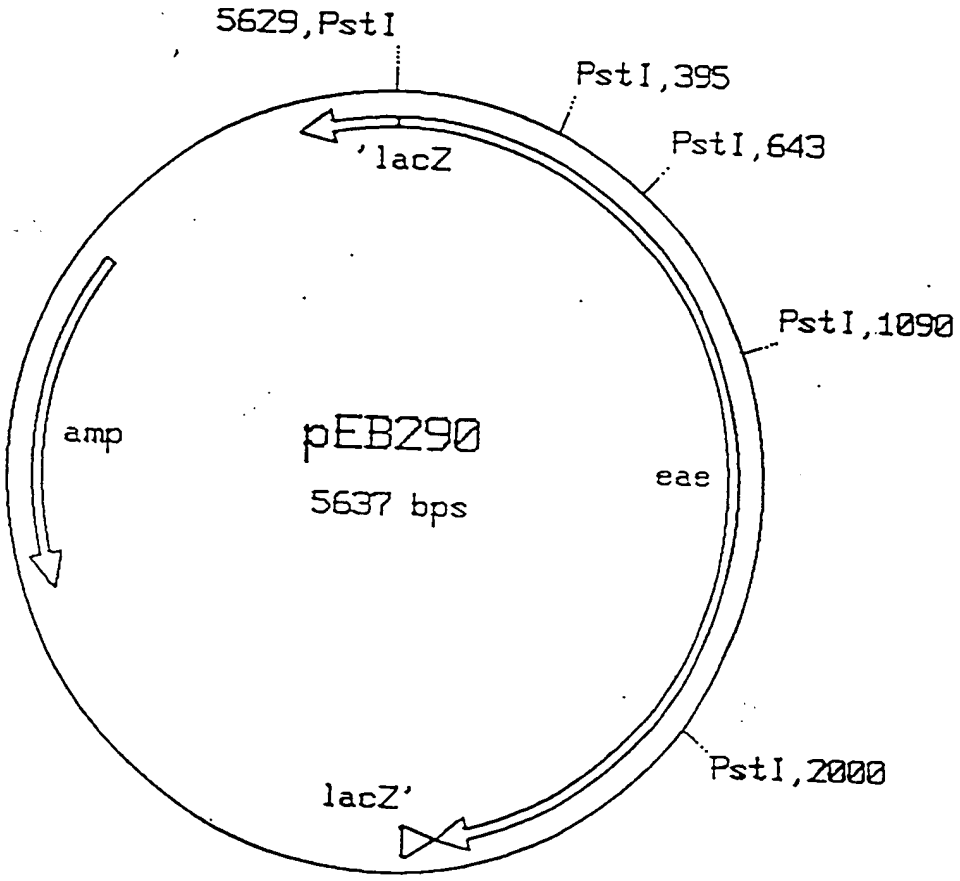
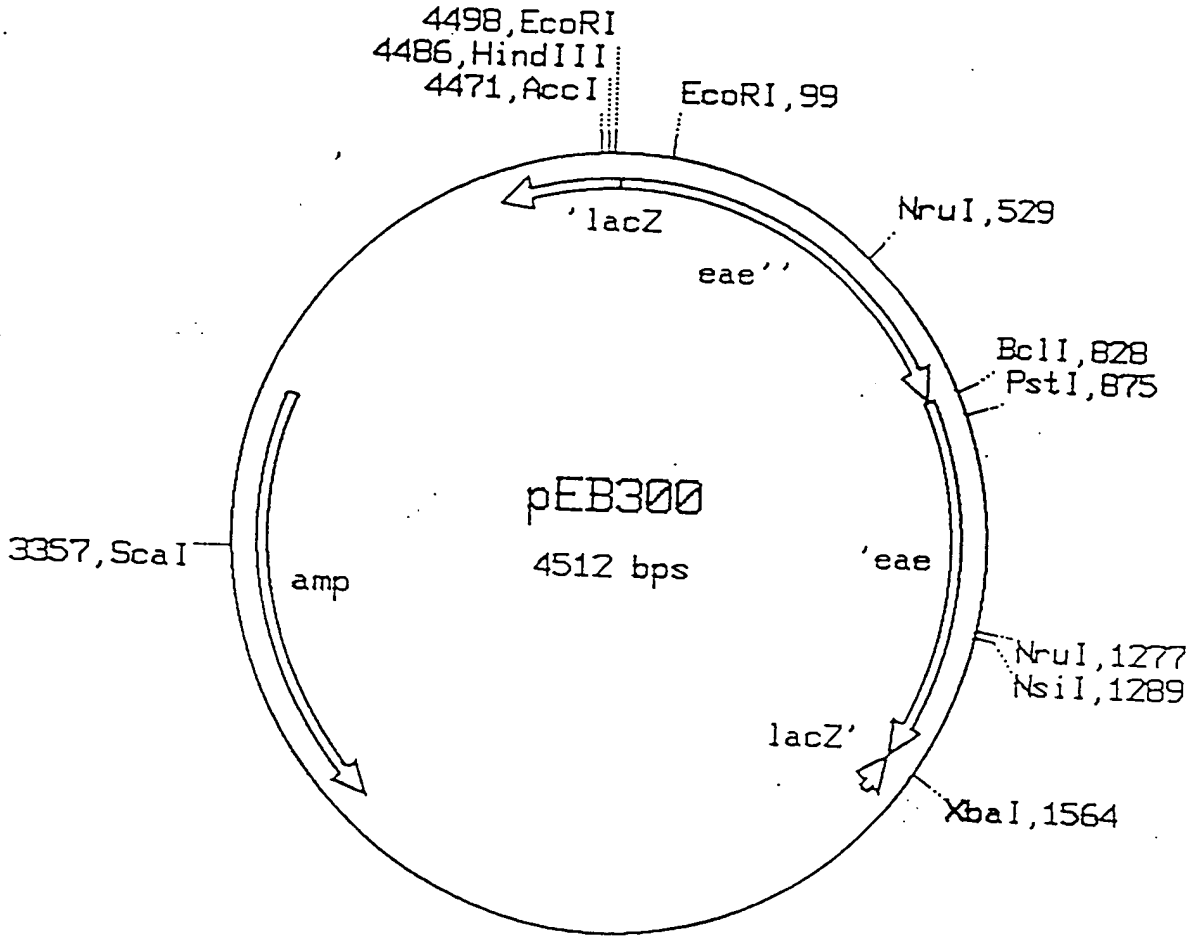



FIGURE 14


4498,EcoRI
4486,HindIII
4471,AccI
EcoRI,99
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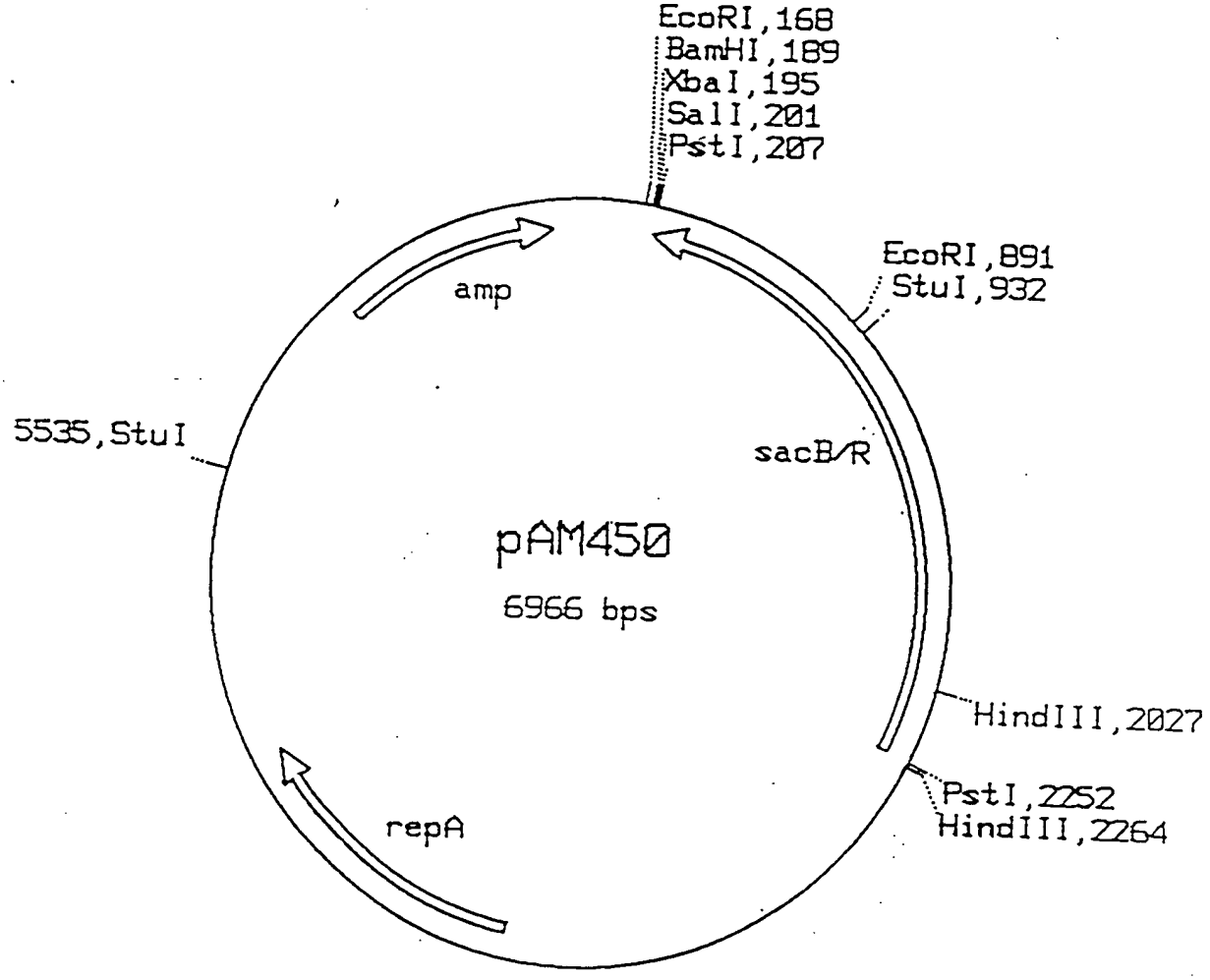
NruI,529
eae''
BclI,828
PstI,875
pEB300
4512 bps
3357,ScaI
amp
'eae
NruI,1277
NsiI,1289
lacZ'
XbaI,1564

FIGURE 15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BamHI,189
XbaI,195
SalI,201
PstI,207
EcoRI,891
StuI,932
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sacB/R
pAM450
6966 bps
HindIII,2027
PstI,2252
HindIII,2264
repA

FIGURE 16

MOORE JAMES SB

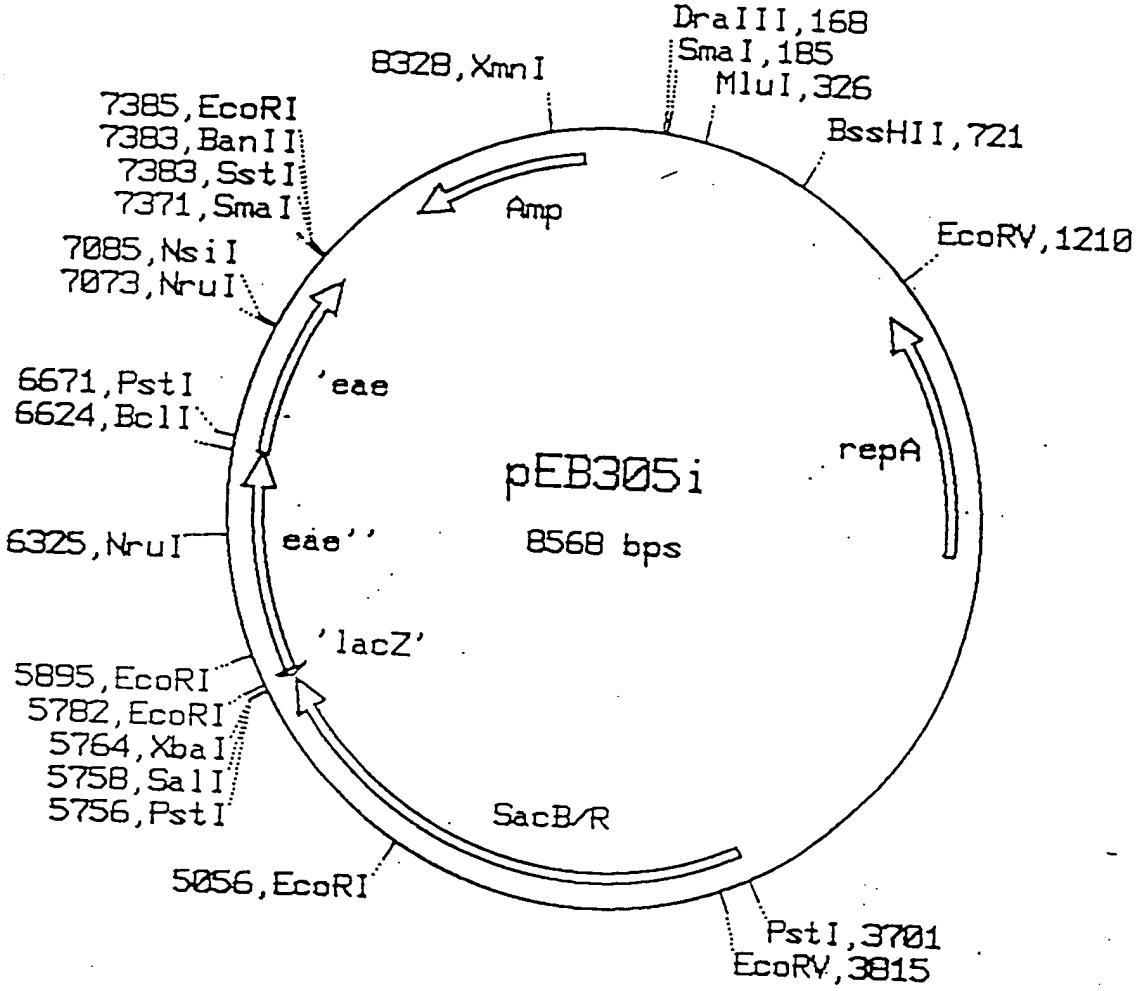


FIGURE 17

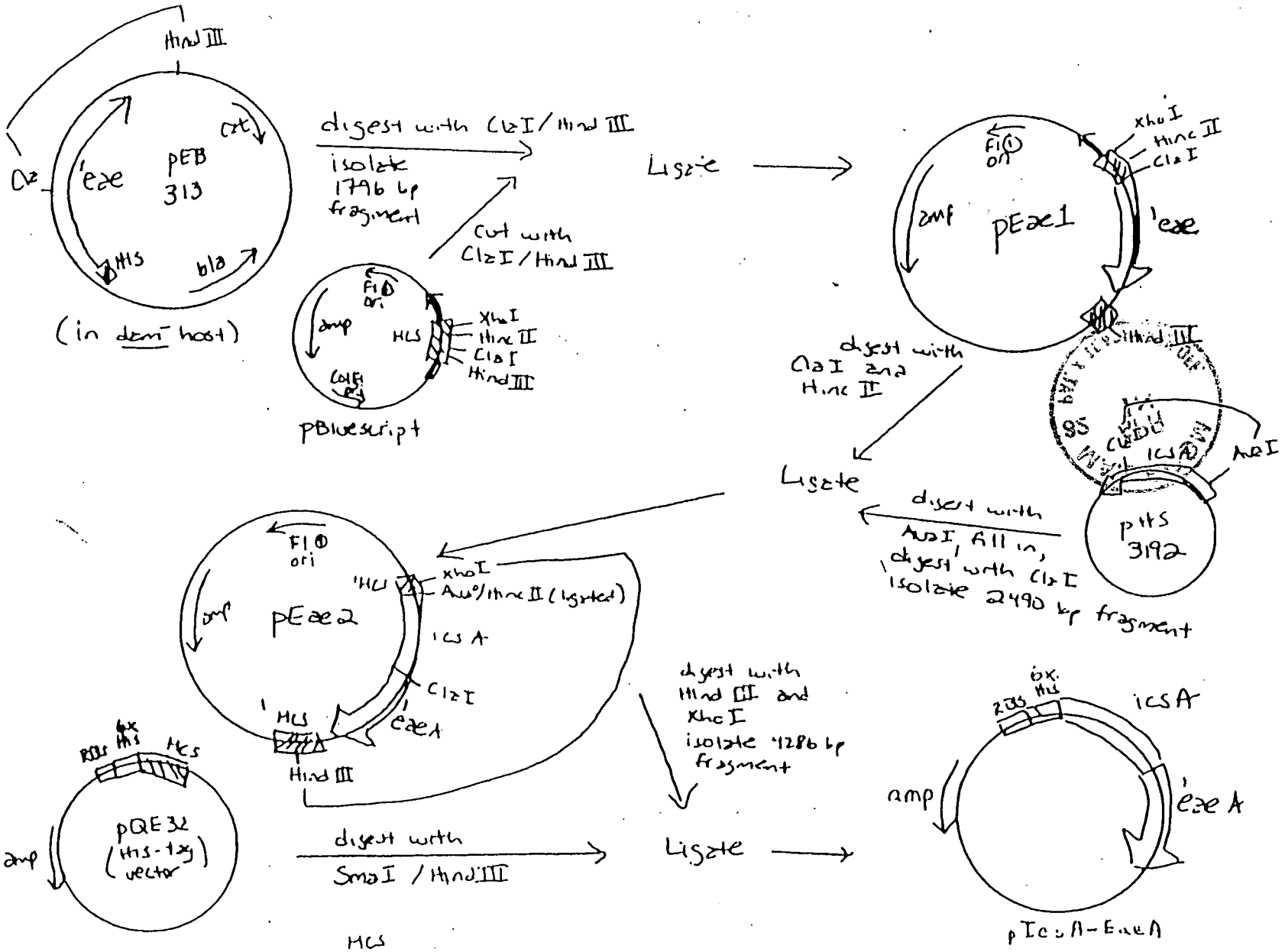
HindIII
ClaI
'eae
pEB 313
cat
HIS
bla
(in dam⁻ host)
digest with ClaI / HindIII
isolate 1796 bp fragment
cut with ClaI / HindIII
pBluescript
F1 ori
amp
HCS
XhoI
HincII
ClaI
HindIII
ColE1
Ligate
pEae1
'eae
NdeI
digest with ClaI and HincII
Ligate
digest with NdeI, fill in, digest with ClaI isolate 2490 bp fragment
pHS 3192
pEae2
'HCS
ICS A
ClaI
'eae1
XhoI
AvaI/HincII (ligated)
digest with HindIII and XhoI isolate 4286 bp fragment
pQE32 (His-tag vector)
RBS
6x His
MCS
digest with SmaI / HindIII
Ligate
6x His
RBS
235
ICS A
amp
'eae A
pICsA-EaeA
MCS = multiple cloning site, RBS = ribosome binding site

FIGURE 18